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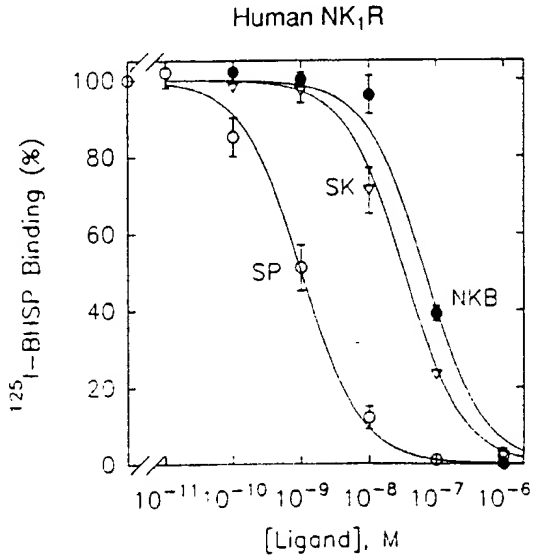
(54) Human neurokinin-1 receptor.

(57) Recombinant human neurokinin-I receptor (human NKIR) is disclosed which has been prepared by polymerase chain reaction techniques. Also disclosed is the complete sequence of human NKIR complementary DNA ; expression systems, including a CHO (chinese hamster ovarian cell line) stable expression system ; and an assay using the CHO expression system.

NKIR, also known as substance P receptor, can be used in an assay to identify and evaluate entities that bind substance P receptor. The assay can also be used in conjunction with diagnosis and therapy to determine the body fluid concentration of substance P in arthritis patients.

FIGURE 1



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BACKGROUND OF THE INVENTION

The present invention concerns cloned human neurokinin-I receptor (human NKIR) and recombinant human NKIR. Neurokinin-I receptor is also known as substance P receptor.

J. Yokota, et al., J. Biol. Chem., 264:17649 (1989) have reported cloned rat neurokinin-I receptor. N.P. Gerard, et al., J. Biol. Chem., 265:20455 (1990), have reported human neurokinin-2 receptor. Cloned rat and bovine neurokinin-2 receptor have likewise been reported. See respectively, Y. Sasi, and S. Nakanishi, Biochem Biophys. Res. Comm., 165:695 (1989), and Y. Masu, et al., Nature 329:836 (1987). Cloned rat neurokinin-3 receptor has also been reported by R. Shigemoto, et al., J. Biol. Chem., 265:623 (1990).

The above references, however, neither disclose or suggest the instant invention. In particular, the pharmacological profile of the human receptor differs significantly from the rat. Moreover, the rat neurokinin-I receptor differs from the NKIR disclosed herein by 23 amino acids.

Substance P is a naturally occuring undecapeptide belonging to the tachykinin family of peptides. Substance P is a pharmacologically-active neuropeptide that is produced in mammals. Its characteristic amino acid sequence is illustrated in U.S. 4,680,283. As is well known in the art substance P and other tachykinins have been implicated in the pathophysiology of numerous diseases. Substance P has been shown to be involved in the transmission of pain or migraine (see B.E.B. Sandberg et al., Journal of Medicinal Chemistry, Vol. 25, p. 1009 (1982)), as well as in central nervous system disorders such as anxiety and schizophrenia, in respiratory and inflammatory diseases such as asthma and rheumatoid arthritis, respectively, and in gastrointestinal disorders and diseases of the GI tract, like ulcerative colitis and Crohn's disease, etc. (see D. Regoli in "Trends in Cluster Headache," edited by F. Sicuteri et al., Elsevier Scientific Publishers, Amsterdam, 1987, pp. 85-95).

The instant invention also concerns an assay protocol which can be used to determine substance P activity in body fluids. The assay can also be used for identifying and evaluating substances that bind substance P receptor. Thus, the assay can be used to identify substance P antagonists and evaluate their binding affinity. Other methods includes that described by M.A. Cascieri, et al., J. Biol. Chem., 258-5158 (1983).
By use of such methods, substance P antagonists have been identified. See, for example, R. M. Snider, et al., Science, 251:435 (Jan. 1991) and S. McLean, et al., Science, 251:437 (Jan. 1991). See also WO90/05525 which published May 31, 1990, which is hereby incorporated by reference. Methods to date have proven inferior, in part, for failure of the animal receptor (animal NKIR, NK2R or NK3R) activity to accurately reflect that of human neurokinin-I receptor. Furthermore, prior to this disclosure human NKIR has not been available in a purified form or in substantial isolation from NK2R and/or NK3R.

Use of such neurokinin receptor sources can not accurately depict the affinity for human NKIR.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure I Full length amino acid sequence of human neurokinin-I receptor.

Figure 2 Full length nucleotide sequence of the cloned human neurokinin-I receptor complementary DNA.

Figure 3 Competitive binding of substance P (SP), substance K (SK) and human neurokinin-I receptor (NKIR) in COS assay.

SUMMARY OF THE INVENTION

Recombinant human neurokinin-I receptor (human NKIR) is disclosed which has been prepared by polymerase chain reaction techniques. Also disclosed is the complete sequence of human NKLR complementary DNA; expression systems, including a CHO (chinese hamster ovarian cell line) stable expression system; and an assay using the CHO expression system.

NKIR, also known as substance P receptor, can be used in an assay to identify and evaluate entities that bind substance P receptor. The assay can also be used in conjunction with diagnosis and therapy to determine the body fluid concentration of substance P in arthritis patients.

DETAILED DESCRIPTION OF THE INVENTION

One embodiment of the invention concerns human neurokinin-I receptor, said receptor being free of other human receptor proteins.

In one class this embodiment concerns human neurokinin-I receptor, said receptor being free of other human proteins.

Within this class, this embodiment concerns human neurokinin-I receptor from human cells such as glioblastoma, said receptor being free of other proteins.

In a second class, this embodiment concerns a protein comprising the 407 amino acid sequence depicted in Figure I, said protein being free of other human receptor proteins.

Within the second class this embodiment concerns a protein consisting of the 407 amino acid sequence as shown in Figure I.

The first embodiment also concerns a pharmaceutical composition for inhibiting the binding of substance P to cellular neueokinin-I receptor, said composition comprising an effective amount ofneurokinin-I receptor.

The first embodiment also concerns a method of inhibiting the binding of substance P to cellular human neurokinin-I receptor, in a patient in need of such inhibition, comprising:
administration of an effective amount of human neurokinin-I receptor.

The use of such pharmaceutical compositions and methods for antagonising the binding of substance P to in vivo neurokinin-I receptor is disclosed in, for example, R. M. Snider, et al., Science, 25I:435 (Jan. I99I); S. McLean, et al., Science, 25I:437 (Jan. I99I); and WO90/05525 which published May 3I, I990, which are hereby incorporated by reference.:

A second embodiment concerns a DNA sequence encoding human neurokinin receptor complementary DNA, said DNA, said sequence being free of other human DNA sequences.

As will be appreciated by those of skill in the art, there is a substantial amount of redundancy in the set of condons which translate specific amino acids. Accordingly, the invention also includes alternative base sequences wherein a codon (or codons) are replaced with another codon, such that the amino acid sequence translated by the DNA sequence remains unchanged. For purposes of this specification, a sequence bearing one or more such replaced codons will be defined as a degenetate variation. Also included are mutations (exchange of individual amino acids) which one of skill in the art would expect to have no effect on functionality, such as valine for leucine, arginine for lysine and asparigine for glutamine.

One class of the second embodiment the invention concerns the nucleotide sequence of complementary DNA, beginning with nucleotide I23 and ending with necleotide I346 as shown in Figure 2.

Within this class of the second embodiment is the DNA sequence that further comprises:

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          10         20         30         40         50         60         70
GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA CAGGACTCTG

          80         90         100        110        120 122
CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG AA
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or a degenerate variation thereof.

The second embodiment the invention concerns the partial nucleotide sequence of complementary DNA, as shown in Figure 2 or a degenerate variation thereof.

A third embodiment of this invention concerns systems for expressing human neurokinin receptor.

One class this third embodiment of the invention comprises:

A plasmid which comprises:

(a) a mammalian expression vector, such as pRcCMV, and

(b) a base sequence encoding human neurokinin-I receptor protein.

Within this class of the third embodiment the neurokinin-I receptor comprises the nucleotide sequence of complementary DNA, beginning with nucleotide I23 and ending with necleotide I346 as shown in Figure 2.

A second class of this third embodiment of the invention concerns a system for the transient expression of human neurokinin-I receptor (NKIR) in a monkey kidney cell line (COS).

A third class of this third embodiment of the invention concerns a system for the expression of human neurokinin-I receptor in a chinese hamster ovarian cell line (CHO), the system comprising a vector comprising human neurokinin receptor (NKIR) cDNA.

Within this class of the third embodiment is is the sub-class wherein the expression system includes

A plasmid which comprises:

(a) a mammalian expression vector, such as pRcCMV, and

(b) a base sequence encoding human neurokinin-I receptor protein.

Within this sub-class the neurokinin-I receptor comprises the nucleotide sequence of complementary DNA, beginning with nucleotide I23 and ending with necleotide I346 as shown in Figure 2. is subclosed into the vector pRcCMV.

A forth embodiment of the invention concerns a method of using any of the above expression systems for determining the binding affinity of a test sample for human neurokinin-I receptor.

In one class this embodiment concerns a method of using a Chinese hamster ovarian cell line, said line

transplanted with a plasmid, which plasmid comprises:

(a) vector pRcCMV, and

(b) the base sequence encoding human neurokinin-I receptor protein, the method which comprises:

(I) expressing human neurokinin-I receptor in said CHO cells;

(2) addition of a test sample to a solution containing ^{125}I-substance P and said cells;

(3) incubating the products of Step (I), wherein said incubation effective for expressing said the human neurokinin-I receptor and effective for competitive binding of said ^{125}I-substance P and said test sample to said human neurokinin-I receptor;

(4) separating said ^{125}I-substance P which is bound to said human neurokinin-I receptor from said ^{125}I-substance P which is not bound;

(5) measuring the radioactivity of said ^{125}I-substance P which is bound to said human neurokinin-I receptor.

In a second class this embodiment concerns a method of using a Chinese hamster ovarian cell line (CHO), said line transplanted with a plasmid which plasmid comprises

(a) vector pRcCMV, and

(b) the base sequence encoding human neurokinin-I receptor protein, the method comprising:

(I) expressing human neurokinin-I receptor in said CHO cells;

(2) equilibrating the product of Step (I) with ^{3}H-myoinositol;

(3) washing the product of Step (2);

(4) incubating the product of Step (3) with a test sample in the presence of I0 mM LiCl, which results in the production of inositol monophosphate;

(5) measuring the inositol monophosphate.

In overview, the present invention describes methods to isolate the human neurokinin-I receptor (human NKIR) complementary DNA (cDNA) without prior knowledge of its protein sequence or gene sequence. Human NKIR is a membrane receptor for the neurotransmitter substance P. Polymerase chain reaction (PCR) technique was utilized for the isolation of human NKIR cDNA. In the approach, the regions of rat NKIR Applicants thought to be similar to human NKIR were identified, oligonucleotide primers corresponding to those region were designed, PCR amplification was carried out to obtain part of the NKIR cDNA from human cells, and its DNA sequence was determined. The remaining part of the human NKIR cDNA was obtained from a human cDNA library utilizing the above sequence information of human NKIR cDNA.

The complete sequence of the human NKIR cDNA was determined, and its encoded protein sequence was deduced. Among other things, such sequence information is useful in the process of developing novel substance P antagonists.

Three heterologous expression systems were used to express the cloned human NKIR cDNA. The Xenopus oocyte expression enables one to determine the biological function of human NKIR. The COS (a monkey kidney cell line) expression can be used to measure the ligand binding properties of human NKIR. The CHO (a Chinese hamster ovarian cell line) stable expression is suitable for natural product screen to identify potential therapeutic agent or other substances that bind to substance P receptor. This cell line can also be used as an assay kit for determining the body fluid concentration of substance P in arthritis patients.

Assay protocols use the heterologously expressed human NKIR for determination of the binding affinity and antagonistic activity of substance P antagonists.

1) Isolation of human NKIR cDNA

To isolate the human NKIR cDNA in the absence of its sequence information, we developed methods to obtain three separate but overlapping cDNA clones in three steps. (i) We have adopted the homologous cloning strategy (Ohara et al., 1989, Proc. Nat. Acad. Sci., 86:5673-5677) to isolate cDNA clones encoding the central core region of human NKIR, with the assumption that the human NKIR sequence is similar to the published sequence (Yokota et al., 1989, J. Biol. Chem., 264:17649-17652) of rat NKIR in certain areas where appropriate PCR primers can be designed. Degenerate primers corresponding to the rat sequence were used in PCR amplification (Mullis and Faloona, 1987, Meth. Enzymol., 155:335) to obtain the cDNA encoding the central trnsmembrane core region of human NKIR from human mRNA. (ii) After determining the sequence of the core region in human NKIR, new primers corresponding to the human sequence were designed and a second homologous PCR amplification was performed using the human primer in the core region with degenerate primers corresponding to the N-terminal sequence of rat NKIR. The cDNA encoding the N-terminal region of human NKIR was thus obtained from human mRNA and its sequence was determined. (iii) An anchored PCR strategy was developed to isolate the cDNA encoding the C-terminal region of human NKIR, in which primers corresponding to the core region of human NKIR were used in combination with a primer corresponding to the se-

quence of a cloning vector to obtain the cDNA from a human cDNA library.

To confirm the authenticity of the cDNA encoding human NKIR, an independent PCR amplification was performed to obtain the full length cDNA in a single step using primers from the 5' and 3' untranslated regions.

2) Expression of the cloned human NKIR

Three expression systems were developed for the cloned human NKIR. An transient expression in Xenopus oocytes resulted from microinjection of in vitro transcribed mRNA from the cloned cDNA (Xenopus Laevis from XENOPUS ONE, Ann Arbor, MI). This system allows the measurement of biological effect of NKIR activation upon ligand binding. Another transient expression in COS (a monkey kidney cell line, ATCC CRL 1651, ATCC Rockville MD) resulted from the transfection of the cloned cDNA under the control of viral promoter into mammalian cells (e.g., COS). The transfected cells are suitable for determination binding affinity of human NKIR for various ligands. Stable expression of human NKIR in mammalian cells (e.g., CHO, a Chinese hamster ovarian cell line, ATCC CRL 9096, ATCC Rockville MD) was achieved after integration of the transfected cDNA into the chromosomes of the host cells. These stable cell lines will constituently express the cloned human NKIR and can be propagated infinitely. Therefore, stable expression system is very useful in large scale drug screen, and can be used to determine substance p concentration in the biopsy sample of patients.

To establish a stable cell line expressing the cloned human NKIR, the cDNA was subcloned into the vector pRcCMV (INVITROGEN).

The electrophysiological assay of human NKIR expressed in Xenopus oocytes was based on the fact that NKIR activates the phospholipase C upon substance P binding, and phospholipase C in turn increases the intracellular calcium concentration through inositol trisphosphate (IP_3) and IP_3-gated calcium channel on intracellular membranes. The calcium increase activates calcium-gated chloride channels on plasma membranes which gives rise to a chloride current measurable by two electrode voltage clamp.

The binding assay of human NKIR expressed in COS or CHO is based on the use of ^{125}I-substance P (^{125}I-SP, from DU PONT, Boston, MA) as a radioactively labeled ligand which compete with unlabeled substance p or any other ligand for binding to the human NKIR. Monolayer cell culture of COS or CHO was dissociated by the non-enzymatic solution (SPECIALTY MEDIA, Lavallette, NJ) and resuspended in appropriate volume of the binding buffer (50 mM Tris pH 7.5, 5 mM $MnCl_2$, 150 mM NaCl, 0.04 mg/ml bacitracin, 0.004 mg/ml leupeptin, 0.2 mg/ml BSA, 0.01 mM phosphoramidon) such that 200 ul of the cell suspension would give rise to about 10,000 cpm of specific ^{125}I-SP binding (approximately 50,000 to 200,000 cells).

The activation of phospholipase C by NKIR can also be measured in CHO cells by determining the accumulation of inositol monophosphate which is a degradation product of IP_3.

In addition to large scale drug screening using the stable CHO cell line expressing the cloned human NKIR, other alternative applications are obvious. For example, the stable cell line can be used in the binding assay to determine the substance p concentration from biopsy samples. The human NKIR protein can also be injected into patients to reduce substance P concentration in some neurogenic inflammatory diseases.

EXAMPLE I

Step A:

In the first step of obtaining the cDNA encoding the central core region of human NKIR, human mRNA was prepared from three human glioblastoma cell lines T98G, CCF-STTGI and U87MG (obtained from the American Type Culture Collection, Rockville, MD) by the FASTTRACK method (INVITROGEN, San Diego, CA). Synthesis of first strand cDNA from 4 ug of human mRNA was initiated by oligo (dT) primers in a total volume of 20 ul according to protocols of the BRL cDNA synthesis system (BRL, LIFE TECHNOLOGIES, Inc., Gaithersburg, MD). Ten ul of the first strand cDNA was used as template with three rat primers (50 pmol rspr2s4, 50 pmol rspr2s4h, and 100 pmol rspr7a2; see Table I for their sequences) in a primary PCR amplification in a total volume of 100 ul according to the GENEAMP protocol (PERKIN ELMER CETUS, Norwalk, CT). Thirty cycles of PCR were performed using the following parameters: 1 min of denaturation at 94°C, 2 min of annealing at 40°C and 4 min of extension at 72°C with 2 sec of auto extension. Ten ul of the primary PCR product was used as template with the same primers in a secondary PCR amplification under the same cycling conditions to further amplify the DNA. Ten ul of the secondary PCR product was used as template with three rat primers (50 pmol rspr2s4, 50 pmol rspr2s4h, 50 pmol rspr7al and 50 pmol rspr7alh) in 30 cycles of tertiary PCR amplification with the following parameters: 1 min of denaturation at 94°C, 2 min of annealing at 45°C, and 4 min of extension at 72°C with 2 sec of auto extension. The tertiary PCR product was analyzed by agarose gel electrophoresis and was found to contain a 600 bp DNA fragment. This DNA fragment was excised from the gel, purified by GENECLEAN

(Bio 101, La Jolla, CA), phosphorylated, and subcloned into Sma I site of the plasmid vector BLUESCRIPT SK+ (STRATAGENE, La Jolla, CA). The DNA sequence was determined by the Sequenase dideoxy chain termination method (USBC, Cleveland, OH). Sequence alignment analysis showed that this cDNA fragment is similar (90% identity at nucleotide level) to the central core region of rat NKIR from amino acid 91 to 280.

STEP B:

After determination of the core region sequence of human NKIR, five antisense primers were synthesized based on the human sequence (hspr3a5, hspr5al, hspr5a2, hspr6al and hspr6a2; see Table II for their sequences). These primers would be used to obtain the N-terminal cDNA sequence of human NKIR. One ug of human glioblastoma mRNA and 6 uM of each of the above primers was used in first strand cDNA synthesis in a total volume of 20 ul according the BRL cDNA synthesis protocols. The cDNA was extracted by phenol-chloroform, precipitated by ethanol and dissolved in 30 ul of water. Ten ul of the cDNA was used as template with two rat primers (50 pmol rsprn and 50 pmol rsprnh) and one human primer (150 pmol hspr3a5) in the primary PCR amplification in a total volume of 100 ul. Thirty cycles were performed with the following parameters: 1 min denaturation at 94°C, 1 min of annealing at 55°C, and 3 min of extension at 72°C. Five ul of the primary PCR product was then used as template with two rat primers (50 pmol rsprn and 50 pmol rsprnh) and one human primer (100 pmol hspr3a4) in 30 cycles of secondary PCR amplification with the same parameters. Two ul of the secondary PCR product was used as template with two rat primers (50 pmol rsprn and 50 pmol rsprnh) and one human primer in 30 cycles of tertiary PCR amplification with the same parameters. The tertiary PCR product was analyzed by agarose gel electrophoresis and was found to contain a 500 bp fragment. This DNA fragment can hybridize with a human oligonucleotide (hspr3a2), indicating it is not a non-specific by-product. This DNA fragment was excised from the gel, purified by GENECLEAN (Bio 101), phosphorylated, and subcloned into Sma I site of the vector Bluescript SK+. DNA sequence analysis revealed that this fragment encodes the human NKIR N-terminal region and it also contains 5' untranslated sequence.

STEP C:

In the third step, an anchored PCR protocol was developed in which the cDNA encoding the C-terminal region of human NKIR was obtained from a cDNA library using sense human primers and a primer corresponding to the vector sequence. Three ug of human glioblastoma mRNA was primed by 2.5 ug of oligo (dT) in the first strand cDNA synthesis in a total volume of 50 ul, followed by second strand cDNA synthesis according the BRL cDNA synthesis protocols. The cDNA product was then heated at 70°C for 10 min. The yield of double stranded cDNA was determined by incorporating 1.25 uM of ^{32}P-α-dCTP as tracer in the reaction. Four ul of T4 DNA polymerase was added to the reaction mixture and incubated at 37°C for 10 min. The reaction was stopped by adding 16 ul of 250 mM EDTA, extracting with phenol/$CHCl_3$, and precipitating with ethanol. The cDNA was dissolved in 50 ul of HE buffer (10 mM HEPES-1mM EDTA). Small size cDNA was removed by the Select-D(RF) SPIN COLUMN (5'TO3', Boulder, CO), and the large size cDNA was precipitated by ethanol and dissolved in 36 ul of water. Four ul of 0.2 M Tris-10 mM spermidine-1 mM EDTA (pH7.5) was added to the tube and heated at 70°C for 1 min. The cDNA was phosphorylated by adding 5 ul of blunt-end kinase buffer (0.5 M Tris pH 9.5, 0.1 M $MgCl_2$2, 50 mM DTT, 50% glycerol), 2.5 ul of 10 mM ATP, 2.5 ul of polynucleotide kinase, and incubating at 37°C for 30 min. The cDNA was extracted by phenol/$CHCl_3$, precipitated by ethanol and ligated to EcoRI linker according to the PROMEGA ECORI linker ligation protocol (PROMEGA, Madison, WI). Linker-ligated cDNA was then ligated to calf intestinal phosphatase-treated EcoRI site of the vector BLUESCRIPT SK+. One ul of the ligated plasmid DNA was used as template in 30 cycles of primary PCR with two human primers (50 pmol hspr6sl and 50 pmol hspr6s2) and 100 pmol of vector-specific primer t3 (obtained from STRATAGENE) with the following parameters: 1 min of denaturation at 94°C, 2 min of annealing at 55°C, and 4 min of extension at 72°C with 2 sec auto extension. One ul of the primary PCR product was used in 30 cycles of secondary PCR amplification with one human primer (100 pmol hspr6s3) and the same vector-specific primer t3 under the same conditions. One ul of the secondary PCR product was used in 30 cycles of tertiary PCR amplification with one human primer (100 pmol hspr6s4) and 100 pmol of vector-specific primer SK (STRATAGENE) under the same conditions. A 780 bp DNA fragment was detected which also hybridized to a human oligo probe hspr6s5. This DNA fragment was excised from the agarose gel, purified by GENECLEAN (BIO 101), phosphorylated, and subcloned into Sma I site of the vector BLUESCRIPT SK+. DNA sequence analysis revealed that it encodes the C-terminal region of human NKIR and contains 3' untranslated sequence.

STEP D:

Since three separate but overlapping cDNA clones encoding human NKIR were isolated above and the possibility of alternative pre-mRNA splicing exists, it is necessary to confirm the authenticity of the full length cDNA sequence by isolating a full length cDNA directly. Based on the above sequence in the untranslated region, primers were synthesized which should give rise to a full length cDNA. Using the PERKIN ELMER CETUS RNA PCR amplification kit (Perkin Elmer Cetus), cDNA was synthesized from l.5 ug of human glioblastoma mRNA in a total volume of 20 ul with 50 pmol of the human primer hspr3uta5. One half of the first strand cDNA was used as template in 30 cycles of primary PCR amplification with two human primers (50 pmol hspr3uta5, 50 pmol hspr5utsl) with the following parameters: l min of denaturation at 94°C, 2 min of annealing at 55°C, and 4 min of extension at 55°C with 2 sec auto extension. Ten ul of the primary PCR product was used as template in 30 cycles of secondary PCR amplification with two human primers (50 pmol hspr3uta6 and 50 pmol hspr5uts2) under the same conditions. A l350 bp DNA fragment was excised from agarose gel, purified by GENECLEAN (BIO l0l), digested with restriction endonucleases with EcoRI and Not I, and subcloned into the vector BLUESCRIPT SK+. DNA sequence analysis confirmed the general structure of the cloned human NKIR cDNA. The sequence of human NKIR CDNA is shown in Figure l.

Table I: Primers based on rat NKIR sequence. The last letter "h" in some primers denotes that human codon bias was incorporated (Lathe, l985, J. Mol. Biol., l83:l-l2). The position number in the rat cDNA sequence was defined by Yokota et al. (J. Biol Chem., l989, 264:l7649-l7652).

Name	Sequence	Position	Direction
rspr2s4	tgcatggctgcattcaat	238 - 255	sense
rspr2s4h	tgcatggctgccttcaa	238 - 254	sense
rspr7a2	acagtagatgatggggttgtacat	918 - 894	antisense
rspr7al	caggtagacctgctggatgaactt	864 - 841	antisense
rspr7alh	caggtacacctgctggatgaactt	864 - 841	antisense
rsprn	atggataacgtccttcctat	1 - 20	sense
rsprnh	atggacaatgtgctgccca	1 - 19	sense

Table II: Primers based on the human NKIR cDNA sequence. Position number is defined in the sequence listing in the text. The nucleotides in parentheses are not present in the human NKIR cDNA; they are restriction sites for subcloning purpose.

Name	Sequence	Position	Direction
hspr3a2	gaagaagttgtggaacttgca	455 - 435	antisense
hspr3a1	catggagtagatactggcgaa	491 - 471	antisense
hspr3a4	ggatgtatgatggccatgta	532 - 513	antisense
hspr3a5	actttggtggctgtggctga	568 - 549	antisense
hspr5a1	atgcatagccaatcaccagca	768 - 748	antisense
hspr5a2	catagtgtgattcccactac	793 - 774	antisense
hspr6a1	tgcacaccacgacaatcatca	888 - 868	antisense
hspr6a2	ttgatgtagggcaggaggaa	943 - 924	antisense
hspr6s1	gcaagtctctgccaagcgcaa	836 - 856	sense
hspr6s2	tgatgattgtcgtggtgtgca	868 - 888	sense
hspr6s3	ttccacatcttcttcctcct	912 - 931	sense
hspr6s4	ctacatcaacccagatctct	935 - 954	sense
hspr6s5	tctctacctgaagaagtt	950 - 967	sense
hspr3uta5	caaggatggaatgttttccct	1499- 1479	antisense
hspr3uta6	(gacatgcggccgc)aacccatactgacccttttt	1478 - 1460	antisense
hspr5uts1	cctcctgtctggctttagaa	16 - 35	sense
hspr5uts2	(gcgcagaattc)gtgtacagatagtaggctt	86 - 105	sense

Expression in Xenopus oocytes

To express the human NKIR cDNA in Xenopus oocytes, the cDNA was cloned into an in vitro transcription vector BLUESCRIPT SK+ (STRATAGENE) which contains the T7 promoter for initiation of T7 RNA polymerase catalyzed RNA synthesis. One ug of linear plasmid DNA which contained the human NKIR cDNA downstream of the T7 promoter was used in the in vitro transcription reaction containing 40 mM Tris pH 7.5, 50 mM NaCl, 8 mM $MgCl_2$, 2 mM spermidine, 0.4 mM CTP, 0.4 mM ATP, 0.4 mM UTP, 0.16 mM GTP, 2.5 ul CAP analog (STRATAGENE), 30 mM DTT, I U RNase Block II (STRATAGENE), 0.83 pmol ^{32}P-α-CTP and 25 U of T7 RNA polymerase. The reaction tube was incubated at 37°C for I hour. Usually 5 ug of RNA was synthesized as quantitated by incorporation of ^{32}P-α-CTP into RNA. After RNA synthesis, the plasmid DNA was removed by adding I0 U of RNase free DNase and I U of RNase Block II. The reaction mixture was extracted by phenol/$CHCl_3$, and the unincorporated nucleotides were removed by the Select-D(RF) spin column (5'TO3'). The RNA transcript was precipitated by ethanol twice and dissolved in RNase free water. Oocytes were removed from Xenopus frogs, treated with 2 mg/ml collagenase (specific activity < 0.3 U/mg, BOEHRINGER MANNHEIM, Indianapolis, IN) in OR-2 buffer (82.5 mM NaCl, 2 mM KCl, I mM $MgCl_2$, 5 mM HEPES, pH 7.4) for 4 hours at I9°C. The dissociated oocytes were incubated in OR-2 buffer supplemented by I.8 mM $CaCl_2$, 0.5 mg/ml gentamycin and 0.5 mM theophylline at I9°C overnight before injection. A 50 nl aliquot contain 2 ng of RNA transcript was injected into each oocyte. The injected oocytes were incubated at I9°C for 2 days before electrophysiological recording (see Example 3 for assay method).

Expression in COS

To express the human NKIR transiently in COS, the cDNA was cloned into the expression vector pCDM9 which was derived from pCDM8 (INVITROGEN) by inserting the ampicillin resistance gene (nucleotide I973 to 2964 from BLUESCRIPT SK+) into the Sac II site. Transfection of 20 ug of the plasmid DNA into I0 millions COS cells was achieved by electroporation in 800 ul of transfection buffer (I35 mM NaCl, I.2 mM $CaCl_2$, I.2 mM $MgCl_2$, 2.4 mM K_2HPO_4, 0.6 mM KH_2PO_4, I0 mM glucose, I0 mM HEPES pH 7.4) at 260 V and 950 uF using the IBI GENEZAPPER (IBI, New Haven, CT). The cells were incubated in I0% fetal calf serum, 2 mM glutamine, I00U/ml penicillin-streptomycin, and 90% DMEM media (GIBCO, Grand Island, NY) in 5% CO_2 at 37°C for three days before the binding assay.

Stable Expression in CHO

To establish a stable cell line expressing the cloned human NKIR, the cDNA was subcloned into the vector pRcCMV (INVITROGEN). Transfection of 20 ug of the plasmid DNA into CHO cells was achieved by electroporation in 800 ul of transfection buffer suplemented with 0.625 mg/ml Herring sperm DNA at 300 V and 950

uF using the IBI GENEZAPPER (IBI). The transfected cells were incubated in CHO media [10 % fetal calf serum, 100 U/ml pennicilin-streptomycin, 2 mM glutamine, 1/500 hypoxanthine-thymidine (ATCC), 90% IMDM media (JRH BIOSCIENCES, Lenexa, KS), 0.7 mg/ml G418 (GIBCO)] in 5% CO_2 at 37°C until colonies were visible. Each colony was separated and propagated. The cell clone with the highest number of human NKIR was selected for subsequent application in the assay of Example 3.

Example 3

Assay Protocol Using Oocytes

The oocyte was voltage-clamped at - 80 mV by the model 8500 intracellular preamp-clamp (DAGAN, Minneapolis, MN). The recoding chamber was continuously perfused with recording buffer (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.4). Chloride current was elicited by applying substance P (from 0.1 nM to 1000 nM) to the recording chamber. At least three oocytes were measured for each concentration. The antagonistic activity of any potential substance P antagonist can be assessed by determining the inhibition of substance P response. Likewise, NKI agonists can be identified by their ability to stimulate a response in oocytes injected with NKIR mRNA but not in uninjected oocytes.

Assay Protocol using COS or CHO

The binding assay of human NKIR expressed in COS or CHO is based on the use of ^{125}I-substance P (^{125}I-SP, from DU PONT, Boston, MA) as a radioactively labeled ligand which compete with unlabeled substance p or any other ligand for binding to the human NKIR. Monolayer cell culture of COS or CHO was dissociated by the non-enzymatic solution (SPECIALTY MEDIA, Lavallette, NJ) and resuspended in appropriate volume of the binding buffer (50 mM Tris pH 7.5, 5 mM $MnCl_2$, 150 mM NaCl, 0.04 mg/ml bacitracin, 0.004 mg/ml leupeptin, 0.2 mg/ml BSA, 0.01 mM phosphoramidon) such that 200 ul of the cell suspension would give rise to about 10,000 cpm of specific ^{125}I-SP binding (approximately 50,000 to 200,000 cells). In the binding assay, 200 ul of cells were added to a tube containing 20 ul of 1.5 to 2.5 nM of ^{125}I-SP and 20 ll of unlabeled substance p or any other test compound. The tubes were incubated at 4°C or at room temperature for 1 hour with gentle shaking. The bound radioactivity was separated from unbound radioactivity by GF/C filter (BRANDEL, Gaithersburg, MD) which was pre-wetted with 0.1 % polyethylenimine. The filter was washed with 3 ml of wash buffer (50 mM Tris pH 7.5, 5 mM $MnCl_2$, 150 mM NaCl) three times and its radioactivity was determined by gamma counter. Illustrative of this method of using these expression systems are the results shoen in Figure 3. These results show the competitive binding of substance P (SP), substance K (SK) and human neurokinin-1 receptor (NKIR) in the COS assay.

ALTERNATIVE PROTOCOL

The activation of phospholipase C by NKLR can also be measured in CHO cells by determining the accumulation of inositol monophosphate which is a degradation product of IP_3. CHO cells were seeded in 12-well plate at 250,000 cells per well. After incubating in CHO media for 4 days, cells were loaded with 0.025 uCi/ml of ^{3}H-myoinositol by overnight incubation. The extracellular radioactivity was removed by washing with phosphate buffered saline. LiCl was added to the well at final concentration of 0.1 mM with or without antagonist, and continued incubation at 37°C for 15 min. Substance p was added to the well at final concentration of 0.3 nM to activate the human NKIR. After 30 min of incubation at 37°C, the media was removed and 0.1 N HCl was added. Each well was sonicated at 4°C and extracted with $CHCl_3$/methanol (1:1). The aqueous phase was applied to a 1 ml Dowex AG 1X8 ion exchange column. The column was washed with 0.1 N formic acid followed by 0.025 M ammonium formate-0.1 N formic acid. The inositol monophosphate was eluted with 0.2 M ammonium formate-0.1 N formic acid and quantitated by beta counter.

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA 50

GGCGCCACGA CAGGACTCTG CTGCAGAGGG GGGTTGTGTA CAGATAGTAG 100

GGCTTTACCG CCTAGCTTCG AA 122

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGCATGGCTG CATTCAAT 18

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGCATGGCTG CCTTCAA 17

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACAGTAGATG ATGGGGTTGT ACAT 24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGGTAGACC TGCTGGATGA ACTT 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGGTACACC TGCTGGATGA ACTT 24

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGATAACG TCCTTCCTAT 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGGACAATG TGCTGCCCA 19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAAGAAGTTG TGGAACTTGC A 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATGGAGTAG ATACTGGCGA A 21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGATGTATGA TGGCCATGTA 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACTTTGGTGG CTGTGGCTGA 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGCATAGCC AATCACCAGC A 21

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CATAGTGTGA TTCCCACTAC 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGCACACCAC GACAATCATC A 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTGATGTAGG GCAGGAGGAA 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCAAGTCTCT GCCAAGCGCA A 21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TGATGATTGT CGTGGTGTGC A 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTCCACATCT TCTTCCTCCT 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTACATCAAC CCAGATCTCT 20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCTCTACCTG AAGAAGTT 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CAAGGATGGA ATGTTTTCCC T 21

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GACATGCGGC CGCAACCCAT ACTGACCCTT TT 32

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CCTCCTGTCT GGCTTTAGAA 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCGCAGAATT CGTGTACAGA TAGTAGGCTT 30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1224 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATGGATAA CGTCCTCCCG 140

GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT CGGAACCCAA TCAGTTCGTG 200

CAACCAGCCT GGCAAATTGT CCTTTGGGCA GCTGCCTACA CGGTCATTGT GGTGACCTCT 260

GTGGTGGGCA ACGTGGTAGT GATGTGGATC ATCTTAGCCC ACAAAAGAAT GAGGACAGTG 320

ACGAACTATT TTCTGGTGAA CCTGGCCTTC GCGGAGGCCT CCATGGCTGC ATTCAATACA 380

GTGGTGAACT TCACCTATGC TGTCCACAAC GAATGGTACT ACGGCCTGTT CTACTGCAAG 440

TTCCACAACT TCTTCCCCAT CGCCGCTGTC TTCGCCAGTA TCTACTCCAT GACGGCTGTG 500

GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC CCCGGCTGTC AGCCACAGCC 560

ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC TGCTGGCCTT CCCCCAGGGC 620

TACTACTCAA CCACAGAGAC CATGCCCAGC AGAGTCGTGT GCATGATCGA ATGGCCAGAG 680

CATCCGAACA AGATTTATGA GAAAGTGTAC CACATCTGTG TGACTGTGCT GATCTACTTC 740

CTCCCCCTGC TGGTGATTGG CTATGCATAC ACCGTAGTGG GAATCACACT ATGGGCCAGT 800

GAGATCCCCG GGGACTCCTC TGACCGCTAC CACGAGCAAG TCTCTGCCAA GCGCAAGGTG 860

GTCAAAATGA TGATTGTCGT GGTGTGCACC TTCGCCATCT GCTGGCTGCC CTTCCACATC 920

TTCTTCCTCC TGCCCTACAT CAACCCAGAT CTCTACCTGA AGAAGTTTAT CCAGCAGGTC 980

TACCTGGCCA TCATGTGGCT GGCCATGAGC TCCACCATGT ACAACCCCAT CATCTACTGC 1040

TGCCTCAATG ACAGGTTCCG TCTGGGCTTC AAGCATGCCT TCCGGTGCTG CCCCTTCATC 1100

AGCGCCGGCG ACTATGAGGG GCTGGAAATG AAATCCACCC GGTATCTCCA GACCCAGGGC 1160

AGTGTGTACA AAGTCAGCCG CCTGGAGACC ACCATCTCCA CAGTGGTGGG GGCCCACGAG 1220

GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCCTCGTCCC TGGACCTGAC CTCCAACTGC 1280

TCTTCACGAA GTGACTCCAA GACCATGACA GAGAGCTTCA GCTTCTCCTC CAATGTGCTC 1340

TCCTAG 1346

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCCA CAGGGCCTTT GGCAGGTGCA GCCCCCACTG CCTTTGACCT 1390

GCCTCCCTTC ATGCATGGAA ATTCCCTTCA TCTGGAACCA TCAGAAACAC CCTCACACTG 1450

GGACTTGCAA AAAGGGTCAG TATGGGTTAG GGAAAACATT CCATCCTTGA GTCAAAAAAT 1510

CTCAATTCTT CCCTATCTTT GCCACCCTCA TGCTGTGTGA CTCAAACCAA ATCACTGAAC 1570

TTTGCTGAGC CTGTAAAATA AAAGGTCGGA CCAGCTTTTC CCAAAAGCCC ATTCATTCCA 1630

TTCTGGAAGT GACTTTGGCT GCATGCGAGT GCTCATTTCA GGATGAATT 1679

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1346 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA 60

CAGGACTCTG CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG 120

AAATGGATAA CGTCCTCCCG GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT 180

CGGAACCCAA TCAGTTCGTG CAACCAGCCT GGCAAATTGT CCTTTGGGCA GCTGCCTACA 240

CGGTCATTGT GGTGACCTCT GTGGTGGGCA ACGTGGTAGT GATGTGGATC ATCTTAGCCC 300

ACAAAAGAAT GAGGACAGTG ACGAACTATT TTCTGGTGAA CCTGGCCTTC GCGGAGGCCT 360

CCATGGCTGC ATTCAATACA GTGGTGAACT TCACCTATGC TGTCCACAAC GAATGGTACT 420

ACGGCCTGTT CTACTGCAAG TTCCACAACT TCTTCCCCAT CGCCGCTGTC TTCGCCAGTA 480

TCTACTCCAT GACGGCTGTG GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC 540

CCCGGCTGTC AGCCACAGCC ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC 600

TGCTGGCCTT CCCCCAGGGC TACTACTCAA CCACAGAGAC CATGCCCAGC AGAGTCGTGT 660

GCATGATCGA ATGGCCAGAG CATCCGAACA AGATTTATGA GAAAGTGTAC CACATCTGTG 720

TGACTGTGCT GATCTACTTC CTCCCCCTGC TGGTGATTGG CTATGCATAC ACCGTAGTGG 780

GAATCACACT ATGGGCCAGT GAGATCCCCG GGGACTCCTC TGACCGCTAC CACGAGCAAG 840

TCTCTGCCAA GCGCAAGGTG GTCAAAATGA TGATTGTCGT GGTGTGCACC TTCGCCATCT 900

GCTGGCTGCC CTTCCACATC TTCTTCCTCC TGCCCTACAT CAACCCAGAT CTCTACCTGA 960

AGAAGTTTAT CCAGCAGGTC TACCTGGCCA TCATGTGGCT GGCCATGAGC TCCACCATGT 1020

ACAACCCCAT CATCTACTGC TGCCTCAATG ACAGGTTCCG TCTGGGCTTC AAGCATGCCT 1080

TCCGGTGCTG CCCCTTCATC AGCGCCGGCG ACTATGAGGG GCTGGAAATG AAATCCACCC 1140

GGTATCTCCA GACCCAGGGC AGTGTGTACA AAGTCAGCCG CCTGGAGACC ACCATCTCCA 1200

CAGTGGTGGG GGCCCACGAG GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCCTCGTCCC 1260

TGGACCTGAC CTCCAACTGC TCTTCACGAA GTGACTCCAA GACCATGACA GAGAGCTTCA 1320

GCTTCTCCTC CAATGTGCTC TCCTAG 1346

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 407 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Met Asp Asn Val Leu Pro Val Asp Ser Asp Leu Ser Pro Asn Ile Ser
1 5 10 15

Thr Asn Thr Ser Glu Pro Asn Gln Phe Val Gln Pro Ala Trp Gln Ile
20 25 30

Val Leu Trp Ala Ala Ala Tyr Thr Val Ile Val Val Thr Ser Val Val
35 40 45

Gly Asn Val Val Val Met Trp Ile Ile Leu Ala His Lys Arg Met Arg
50 55 60

Thr Val Thr Asn Tyr Phe Leu Val Asn Leu Ala Phe Ala Glu Ala Ser
65 70 75 80

Met Ala Ala Phe Asn Thr Val Val Asn Phe Thr Tyr Ala Val His Asn
85 90 95

Glu Trp Tyr Tyr Gly Leu Phe Tyr Cys Lys Phe His Asn Phe Phe Pro
100 105 110

Ile Ala Ala Val Phe Ala Ser Ile Tyr Ser Met Thr Ala Val Ala Phe
115 120 125

Asp Arg Tyr Met Ala Ile Ile His Pro Leu Gln Pro Arg Leu Ser Ala
130 135 140

Thr Ala Thr Lys Val Val Ile Cys Val Ile Trp Val Leu Ala Leu Leu
145 150 155 160

Leu Ala Phe Pro Gln Gly Tyr Tyr Ser Thr Thr Glu Thr Met Pro Ser
165 170 175

Arg Val Val Cys Met Ile Glu Trp Pro Glu His Pro Asn Lys Ile Tyr
180 185 190

Glu Lys Val Tyr His Ile Cys Val Thr Val Leu Ile Tyr Phe Leu Pro
195 200 205

Leu Leu Val Ile Gly Tyr Ala Tyr Thr Val Val Gly Ile Thr Leu Trp
210 215 220

Ala Ser Glu Ile Pro Gly Asp Ser Ser Asp Arg Tyr His Glu Gln Val
225 230 235 240

Ser Ala Lys Arg Lys Val Val Lys Met Met Ile Val Val Val Cys Thr
245 250 255

Phe Ala Ile Cys Trp Leu Pro Phe His Ile Phe Phe Leu Leu Pro Tyr
260 265 270

Ile Asn Pro Asp Leu Tyr Leu Lys Lys Phe Ile Gln Gln Val Tyr Leu
275 280 285

Ala Ile Met Trp Leu Ala Met Ser Ser Thr Met Tyr Asn Pro Ile Ile
290 295 300

Tyr Cys Cys Leu Asn Asp Arg Phe Arg Leu Gly Phe Lys His Ala Phe
305 310 315 320

Arg Cys Cys Pro Phe Ile Ser Ala Gly Asp Tyr Glu Gly Leu Glu Met
325 330 335

Lys Ser Thr Arg Tyr Leu Gln Thr Gln Gly Ser Val Tyr Lys Val Ser
340 345 350

Arg Leu Glu Thr Thr Ile Ser Thr Val Val Gly Ala His Glu Glu Glu
355 360 365

Pro Glu Asp Gly Pro Lys Ala Thr Pro Ser Ser Leu Asp Leu Thr Ser
370 375 380

Asn Cys Ser Ser Arg Ser Asp Ser Lys Thr Met Thr Glu Ser Phe Ser
385 390 395 400

Phe Ser Ser Asn Val Leu Ser
405

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1679 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA 60

CAGGACTCTG CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG 120

AAATGGATAA CGTCCTCCCG GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT 180

CGGAACCCAA TCAGTTCGTG CAACCAGCCT GGCAAATTGT CCTTTGGGCA GCTGCCTACA 240

CGGTCATTGT GGTGACCTCT GTGGTGGGCA ACGTGGTAGT GATGTGGATC ATCTTAGCCC 300

ACAAAAGAAT GAGGACAGTG ACGAACTATT TTCTGGTGAA CCTGGCCTTC GCGGAGGCCT 360

CCATGGCTGC ATTCAATACA GTGGTGAACT TCACCTATGC TGTCCACAAC GAATGGTACT 420

ACGGCCTGTT CTACTGCAAG TTCCACAACT TCTTCCCCAT CGCCGCTGTC TTCGCCAGTA 480

TCTACTCCAT GACGGCTGTG GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC 540

CCCGGCTGTC AGCCACAGCC ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC 600

TGCTGGCCTT CCCCCAGGGC TACTACTCAA CCACAGAGAC CATGCCCAGC AGAGTCGTGT 660

GCATGATCGA ATGGCCAGAG CATCCGAACA AGATTTATGA GAAAGTGTAC CACATCTGTG 720

TGACTGTGCT GATCTACTTC CTCCCCCTGC TGGTGATTGG CTATGCATAC ACCGTAGTGG 780

GAATCACACT ATGGGCCAGT GAGATCCCCG GGGACTCCTC TGACCGCTAC CACGAGCAAG 840

TCTCTGCCAA GCGCAAGGTG GTCAAAATGA TGATTGTCGT GGTGTGCACC TTCGCCATCT 900

GCTGGCTGCC CTTCCACATC TTCTTCCTCC TGCCCTACAT CAACCCAGAT CTCTACCTGA 960

AGAAGTTTAT CCAGCAGGTC TACCTGGCCA TCATGTGGCT GGCCATGAGC TCCACCATGT 1020

ACAACCCCAT CATCTACTGC TGCCTCAATG ACAGGTTCCG TCTGGGCTTC AAGCATGCCT 1080

TCCGGTGCTG CCCCTTCATC AGCGCCGGCG ACTATGAGGG GCTGGAAATG AAATCCACCC 1140

GGTATCTCCA GACCCAGGGC AGTGTGTACA AAGTCAGCCG CCTGGAGACC ACCATCTCCA 1200

CAGTGGTGGG GGCCCACGAG GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCCTCGTCCC 1260

TGGACCTGAC CTCCAACTGC TCTTCACGAA GTGACTCCAA GACCATGACA GAGAGCTTCA 1320

GCTTCTCCTC CAATGTGCTC TCCTAGGCCA CAGGGCCTTT GGCAGGTGCA GCCCCCACTG 1380

CCTTTGACCT GCCTCCCTTC ATGCATGGAA ATTCCCTTCA TCTGGAACCA TCAGAAACAC 1440

CCTCACACTG GGACTTGCAA AAAGGGTCAG TATGGGTTAG GGAAAACATT CCATCCTTGA 1500

GTCAAAAAAT CTCAATTCTT CCCTATCTTT GCCACCCTCA TGCTGTGTGA CTCAAACCAA 1560

ATCACTGAAC TTTGCTGAGC CTGTAAAATA AAAGGTCGGA CCAGCTTTTC CCAAAAGCCC 1620

ATTCATTCCA TTCTGGAAGT GACTTTGGCT GCATGCGAGT GCTCATTTCA GGATGAATT 1679

Claims

1. A human neurokinin-I receptor, the receptor being substantially free of other-human receptor proteins.

2. A protein corresponding to the amino acid sequence of human neurokinin-I receptor, the protein consisting of 407 amino acids.

3. A protein corresponding to the amino acid sequence which is:

```
                                    10                                  20
Met Asp Asn Val Leu Pro Val Asp Ser Asp Leu Ser Pro Asn Ile Ser Thr Asn Thr Ser

                                    30                                  40
Glu Pro Asn Gln Phe Val Gln Pro Ala Trp Gln Ile Val Leu Trp Ala Ala Ala Tyr Thr

                                    50                                  60
Val Ile Val Val Thr Ser Val Val Gly Asn Val Val Val Met Trp Ile Ile Leu Ala His

                                    70                                  80
Lys Arg Met Arg Thr Val Thr Asn Tyr Phe Leu Val Asn Leu Ala Phe Ala Glu Ala Ser

                                    90                                  100
Met Ala Ala Phe Asn Thr Val Val Asn Phe Thr Tyr Ala Val His Asn Glu Trp Tyr Tyr
```

110 120
Gly Leu Phe Tyr Cys Lys Phe His Asn Phe Phe Pro Ile Ala Ala Val Phe Ala Ser Ile

130 140
Tyr Ser Met Thr Ala Val Ala Phe Asp Arg Tyr Met Ala Ile Ile His Pro Leu Gln Pro

150 160
Arg Leu Ser Ala Thr Ala Thr Lys Val Val Ile Cys Val Ile Trp Val Leu Ala Leu Leu

170 180
Leu Ala Phe Pro Gln Gly Tyr Tyr Ser Thr Thr Glu Thr Met Pro Ser Arg Val Val Cys

190 200
Met Ile Glu Trp Pro Glu His Pro Asn Lys Ile Tyr Glu Lys Val Tyr His Ile Cys Val

210 220
Thr Val Leu Ile Tyr Phe Leu Pro Leu Leu Val Ile Gly Tyr Ala Tyr Thr Val Val Gly

230 240
Ile Thr Leu Trp Ala Ser Glu Ile Pro Gly Asp Ser Ser Asp Arg Tyr His Glu Gln Val

250 260
Ser Ala Lys Arg Lys Val Val Lys Met Met Ile Val Val Val Cys Thr Phe Ala Ile Cys

270 280
Trp Leu Pro Phe His Ile Phe Phe Leu Leu Pro Tyr Ile Asn Pro Asp Leu Tyr Leu Lys

290 300
Lys Phe Ile Gln Gln Val Tyr Leu Ala Ile Met Trp Leu Ala Met Ser Ser Thr Met Tyr

310 320
Asn Pro Ile Ile Tyr Cys Cys Leu Asn Asp Arg Phe Arg Leu Gly Phe Lys His Ala Phe

330 340
Arg Cys Cys Pro Phe Ile Ser Ala Gly Asp Tyr Glu Gly Leu Glu Met Lys Ser Thr Arg

350 360
Tyr Leu Gln Thr Gln Gly Ser Val Tyr Lys Val Ser Arg Leu Glu Thr Thr Ile Ser Thr

370 380
Val Val Gly Ala His Glu Glu Glu Pro Glu Asp Gly Pro Lys Ala Thr Pro Ser Ser Leu

390 400
Asp Leu Thr Ser Asn Cys Ser Ser Arg Ser Asp Ser Lys Thr Met Thr Glu Ser Phe Ser

407
Phe Ser Ser Asn Val Leu Ser

4. A DNA sequence, encoding human neurokin-I receptor, the sequence being free of other human DNA sequences.

5. A DNA sequence comprising the sequence which is:

```
                                                                130         140
                                                               ATGGATAA CGTCCTCCCG

          150         160         170         180         190         200         210
GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT CGGAACCCAA TCAGTTCGTG CAACCAGCCT

          220         230         240         250         260         270         280
GGCAAATTGT CCTTTGGGCA GCTGCCTACA CGGTCATTGT GGTGACCTCT GTGGTGGGCA ACGTGGTAGT

          290         300         310         320         330         340         350
GATGTGGATC ATCTTAGCCC ACAAAAGAAT GAGGACAGTG ACGAACTATT TTCTGGTGAA CCTGGCCTTC

          360         370         380         390         400         410         420
GCGGAGGCCT CCATGGCTGC ATTCAATACA GTGGTGAACT TCACCTATGC TGTCCACAAC GAATGGTACT

          430         440         450         460         470         480         490
ACGGCCTGTT CTACTGCAAG TTCCACAACT TCTTCCCCAT CGCCGCTGTC TTCGCCAGTA TCTACTCCAT

          500         510         520         530         540         550         560
GACGGCTGTG GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC CCCGGCTGTC AGCCACAGCC

          570         580         590         600         610         620         630
ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC TGCTGGCCTT CCCCCAGGGC TACTACTCAA

          640         650         660         670         680         690         700
CCACAGAGAC CATGCCCAGC AGAGTCGTGT GCATGATCGA ATGGCCAGAG CATCCGAACA AGATTTATGA

          710         720         730         740         750         760         770
GAAAGTGTAC CACATCTGTG TGACTGTGCT GATCTACTTC CTCCCCCTGC TGGTGATTGG CTATGCATAC

          780         790         800         810         820         830         840
ACCGTAGTGG GAATCACACT ATGGGCCAGT GAGATCCCCG GGGACTCCTC TGACCGCTAC CACGAGCAAG

          850         860         870         880         890         900         910
TCTCTGCCAA GCGCAAGGTG GTCAAAATGA TGATTGTCGT GGTGTGCACC TTCGCCATCT GCTGGCTGCC

          920         930         940         950         960         970         980
CTTCCACATC TTCTTCCTCC TGCCCTACAT CAACCCAGAT CTCTACCTGA AGAAGTTTAT CCAGCAGGTC

          990         1000        1010        1020        1030        1040        1050
TACCTGGCCA TCATGTGGCT GGCCATGAGC TCCACCATGT ACAACCCCAT CATCTACTGC TGCCTCAATG

          1060        1070        1080        1090        1100        1110        1120
ACAGGTTCCG TCTGGGCTTC AAGCATGCCT TCCGGTGCTG CCCCTTCATC AGCGCCGGCG ACTATGAGGG

          1130        1140        1150        1160        1170        1180        1190
GCTGGAAATG AAATCCACCC GGTATCTCCA GACCCAGGGC AGTGTGTACA AAGTCAGCCG CCTGGAGACC

          1200        1210        1220        1230        1240        1250        1260
ACCATCTCCA CAGTGGTGGG GGCCCACGAG GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCCTCGTCCC

          1270        1280        1290        1300        1310        1320        1330
TGGACCTGAC CTCCAACTGC TCTTCACGAA GTGACTCCAA GACCATGACA GAGAGCTTCA GCTTCTCCTC

          1340      1346
CAATGTGCTC TCCTAG
```

or a degenerate variation thereof.

6. The DNA sequence of Claim 5 further comprising the sequence which is:

```
          10         20         30         40         50         60         70
GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA CAGGACTCTG

          80         90        100        110        120 122
CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG AA
```

or a degenerate variation thereof.

7. The DNA sequence of Claim 6 further comprising the sequence which is:

```
                  1350       1360       1370       1380       1390       1400
                  GCCA CAGGGCCTTT GGCAGGTGCA GCCCCCACTG CCTTTGACCT GCCTCCCTTC

        1410       1420       1430       1440       1450       1460       1470
ATGCATGGAA ATTCCCTTCA TCTGGAACCA TCAGAAACAC CCTCACACTG GGACTTGCAA AAAGGGTCAG

        1480       1490       1500       1510       1520       1530       1540
TATGGGTTAG GGAAAACATT CCATCCTTGA GTCAAAAAAT CTCAATTCTT CCCTATCTTT GCCACCCTCA

        1550       1560       1570       1580       1590       1600       1610
TGCTGTGTGA CTCAAACCAA ATCACTGAAC TTTGCTGAGC CTGTAAAATA AAAGGTCGGA CCAGCTTTTC

        1620       1630       1640       1650       1660       1670       1679
CCAAAAGCCC ATTCATTCCA TTCTGGAAGT GACTTTGGCT GCATGCGAGT GCTCATTTCA GGATGAATT
```

or a degenerate variation thereof.

8. A plasmid which comprises:
   (a) a mammalian expression vector, and
   (b) a base sequence encoding human neurokinin-I receptor protein.

9. A method of using a Chinese hamster ovarian cell line (CHO), the cell line transplanted with a plasmid, which plasmid comprises:
   (a) a mammalian expression vector, and
   (b) a base sequence encoding human neurokinin-I receptor protein,
   the method which comprises:
   (I) expressing human neurokinin-I receptor in the CHO cells;
   (2) adding a test sample to a solution containing ^{125}I-substance P and the CHO cells;
   (3) incubating the products of Step 2, wherein the incubation is effective for competitive binding of the ^{125}I-substance P and the test sample to the human neurokinin-I receptor;
   (4) separating the ^{125}I-substance P which is bound to the human neurokinin-I receptor from the ^{125}I-substance P which is not bound;
   (4) measuring the amount of the ^{125}I-substance P which is bound to the human neurokinin-I receptor.

10. A method of using a Chinese hamster ovarian cell line (CHO), the line transplanted with a plasmid, which plasmid comprises:
   (a) a mammalian expression vector, and
   (b) a base sequence encoding human neurokinin-I receptor protein,
   the method which comprises:
   (I) expressing human neurokinin-I receptor in the CHO cells;
   (2) equilibrating the product of Step (I) with ^{3}H-myoinositol;
   (3) washing the product of Step (2);
   (4) incubating the product of Step (3) with a test sample in the presence of aqueous LiCl, resulting in the production of ^{3}H-inositol monophosphate;
   (5) measuring the ^{3}H-inositol monophosphate.

FIGURE 1

```
                                    10                                  20
Met Asp Asn Val Leu Pro Val Asp Ser Asp Leu Ser Pro Asn Ile Ser Thr Asn Thr Ser

                                    30                                  40
Glu Pro Asn Gln Phe Val Gln Pro Ala Trp Gln Ile Val Leu Trp Ala Ala Ala Tyr Thr

                                    50                                  60
Val Ile Val Val Thr Ser Val Val Gly Asn Val Val Val Met Trp Ile Ile Leu Ala His

                                    70                                  80
Lys Arg Met Arg Thr Val Thr Asn Tyr Phe Leu Val Asn Leu Ala Phe Ala Glu Ala Ser

                                    90                                  100
Met Ala Ala Phe Asn Thr Val Val Asn Phe Thr Tyr Ala Val His Asn Glu Trp Tyr Tyr

                                    110                                 120
Gly Leu Phe Tyr Cys Lys Phe His Asn Phe Phe Pro Ile Ala Ala Val Phe Ala Ser Ile

                                    130                                 140
Tyr Ser Met Thr Ala Val Ala Phe Asp Arg Tyr Met Ala Ile Ile His Pro Leu Gln Pro

                                    150                                 160
Arg Leu Ser Ala Thr Ala Thr Lys Val Val Ile Cys Val Ile Trp Val Leu Ala Leu Leu

                                    170                                 180
Leu Ala Phe Pro Gln Gly Tyr Tyr Ser Thr Thr Glu Thr Met Pro Ser Arg Val Val Cys

                                    190                                 200
Met Ile Glu Trp Pro Glu His Pro Asn Lys Ile Tyr Glu Lys Val Tyr His Ile Cys Val

                                    210                                 220
Thr Val Leu Ile Tyr Phe Leu Pro Leu Leu Val Ile Gly Tyr Ala Tyr Thr Val Val Gly

                                    230                                 240
Ile Thr Leu Trp Ala Ser Glu Ile Pro Gly Asp Ser Ser Asp Arg Tyr His Glu Gln Val

                                    250                                 260
Ser Ala Lys Arg Lys Val Val Lys Met Met Ile Val Val Val Cys Thr Phe Ala Ile Cys
```

FIGURE 1 (continued)

```
                                    270                                 280
Trp Leu Pro Phe His Ile Phe Phe Leu Leu Pro Tyr Ile Asn Pro Asp Leu Tyr Leu Lys

                                    290                                 300
Lys Phe Ile Gln Gln Val Tyr Leu Ala Ile Met Trp Leu Ala Met Ser Ser Thr Met Tyr

                                    310                                 320
Asn Pro Ile Ile Tyr Cys Cys Leu Asn Asp Arg Phe Arg Leu Gly Phe Lys His Ala Phe

                                    330                                 340
Arg Cys Cys Pro Phe Ile Ser Ala Gly Asp Tyr Glu Gly Leu Glu Met Lys Ser Thr Arg

                                    350                                 360
Tyr Leu Gln Thr Gln Gly Ser Val Tyr Lys Val Ser Arg Leu Glu Thr Thr Ile Ser Thr

                                    370                                 380
Val Val Gly Ala His Glu Glu Glu Pro Glu Asp Gly Pro Lys Ala Thr Pro Ser Ser Leu

                                    390                                 400
Asp Leu Thr Ser Asn Cys Ser Ser Arg Ser Asp Ser Lys Thr Met Thr Glu Ser Phe Ser

                                407
Phe Ser Ser Asn Val Leu Ser
```

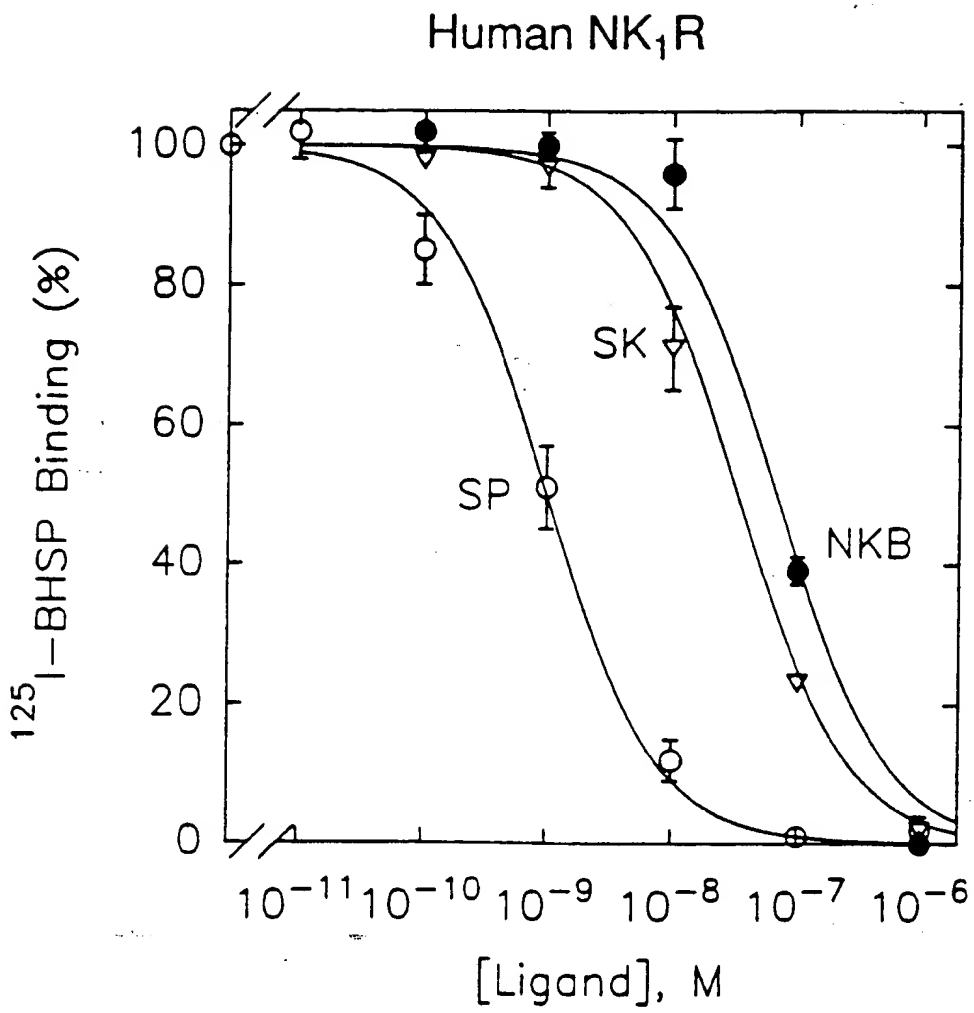
FIGURE 2

```
        10         20         30         40         50         60         70
GAAAAAGCCT TCCACCCTCC TGTCTGGCTT TAGAAGGACC CTGAGCCCCA GGCGCCACGA CAGGACTCTG

        80         90        100        110        120        130        140
CTGCAGAGGG GGGTTGTGTA CAGATAGTAG GGCTTTACCG CCTAGCTTCG AAATGGATAA CGTCCTCCCG

       150        160        170        180        190        200        210
GTGGACTCAG ACCTCTCCCC AAACATCTCC ACTAACACCT CGGAACCCAA TCAGTTCGTG CAACCAGCCT

       220        230        240        250        260        270        280
GGCAAATTGT CCTTTGGGCA GCTGCCTACA CGGTCATTGT GGTGACCTCT GTGGTGGGCA ACGTGGTAGT

       290        300        310        320        330        340        350
GATGTGGATC ATCTTAGCCC ACAAAAGAAT GAGGACAGTG ACGAACTATT TTCTGGTGAA CCTGGCCTTC

       360        370        380        390        400        410        420
GCGGAGGCCT CCATGGCTGC ATTCAATACA GTGGTGAACT TCACCTATGC TGTCCACAAC GAATGGTACT

       430        440        450        460        470        480        490
ACGGCCTGTT CTACTGCAAG TTCCACAACT TCTTCCCCAT CGCCGCTGTC TTCGCCAGTA TCTACTCCAT

       500        510        520        530        540        550        560
GACGGCTGTG GCCTTTGATA GGTACATGGC CATCATACAT CCCCTCCAGC CCCGGCTGTC AGCCACAGCC

       570        580        590        600        610        620        630
ACCAAAGTGG TCATCTGTGT CATCTGGGTC CTGGCTCTCC TGCTGGCCTT CCCCCAGGGC TACTACTCAA

       640        650        660        670        680        690        700
CCACAGAGAC CATGCCCAGC AGAGTCGTGT GCATGATCGA ATGGCCACAG CATCCGAACA AGATTTATGA

       710        720        730        740        750        760        770
GAAAGTGTAC CACATCTGTG TGACTGTGCT GATCTACTTC CTCCCCCTGC TGGTGATTGG CTATGCATAC

       780        790        800        810        820        830        840
ACCGTAGTGG GAATCACACT ATGGGCCAGT GAGATCCCCG GCGACTCCTC TGACCGCTAC CACGAGCAAG

       850        860        870        880        890        900        910
TCTCTGCCAA GCGCAAGGTG GTCAAAATGA TGATTGTCGT GGTGTGCACC TTCGCCATCT GCTGGCTGCC
```

FIGURE 2 (continued)

```
       920        930        940        950        960        970        980
CTTCCACATC TTCTTCCTCC TGCCCTACAT CAACCCAGAT CTCTACCTGA AGAAGTTTAT CCAGCAGGTC

       990       1000       1010       1020       1030       1040       1050
TACCTGGCCA TCATGTGGCT GGCCATGAGC TCCACCATGT ACAACCCCAT CATCTACTGC TGCCTCAATG

      1060       1070       1080       1090       1100       1110       1120
ACAGGTTCCG TCTGGGCTTC AAGCATGCCT TCCGGTGCTG CCCCTTCATC AGCGCCGGCG ACTATGAGGG

      1130       1140       1150       1160       1170       1180       1190
GCTGGAAATG AAATCCACCC GGTATCTCCA GACCCAGGGC AGTGTGTACA AAGTCAGCCG CCTGGAGACC

      1200       1210       1220       1230       1240       1250       1260
ACCATCTCCA CAGTGGTGGC GGCCCACGAG GAGGAGCCAG AGGACGGCCC CAAGGCCACA CCCTCGTCCC

      1270       1280       1290       1300       1310       1320       1330
TGGACCTGAC CTCCAACTGC TCTTCACGAA GTGACTCCAA GACCATGACA GAGAGCTTCA GCTTCTCCTC

      1340       1350       1360       1370       1380       1390       1400
CAATGTGCTC TCCTAGGCCA CAGGGCCTTT GGCAGGTGCA GCCCCCACTG CCTTTGACCT GCCTCCCTTC

      1410       1420       1430       1440       1450       1460       1470
ATGCATGGAA ATTCCCTTCA TCTGGAACCA TCAGAAACAC CCTCACACTG GGACTTGCAA AAAGGGTCAG

      1480       1490       1500       1510       1520       1530       1540
TATGGGTTAG GGAAAACATT CCATCCTTGA GTCAAAAAAT CTCAATTCTT CCCTATCTTT GCCACCCTCA

      1550       1560       1570       1580       1590       1600       1610
TGCTGTGTGA CTCAAACCAA ATCACTGAAC TTTGCTGAGC CTGTAAAATA AAAGGTCGGA CCAGCTTTTC

      1620       1630       1640       1650       1660       1670       1679
CCAAAAGCCC ATTCATTCCA TTCTGGAAGT GACTTTGGCT GCATGCCAGT GCTCATTTCA GGATGAATT
```

FIGURE 3


Human NK1R
125I–BHSP Binding (%)
100
80
60
40
20
0
SK
SP
NKB
10−11 10−10 10−9 10−8 10−7 10−6
[Ligand], M

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92 30 3457

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P,X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 180, no. 2, 31 October 1991, DULUTH, MINNESOTA US pages 1110 - 1117; Hopkins, B. et al.: 'Isolation and characterization of the human lung NK-1 receptor cDNA.' * the whole document * ---	1-10	C12N15/12 C07K13/00 G01N33/58 G01N33/68
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-/--

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	16 JULY 1992	NAUCHE S.A.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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EUROPEAN SEARCH REPORT

Application Number

EP 92 30 3457

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X	JOURNAL OF BIOLOGICAL CHEMISTRY. vol. 264, no. 30, 25 October 1990, BALTIMORE US pages 17649 - 17652; Yokota Y; Sasai Y; Tanaka K; Fujiwara T; Tsuchida K; Shigemoto R; Kakizuka A; Ohkubo H; Nakanishi S: 'Molecular characterization of a functional cDNA for rat substance P receptor.' * the whole document * ---	1-10	
P,X	PATENT ABSTRACTS OF JAPAN vol. 015, no. 341 29 August 1991 & JP-A-30 133 998 (MITSUBISHI KASEI CORP) 7 June 1991 * abstract * -----	1-8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	16 JULY 1992	NAUCHE S.A.

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& : member of the same patent family, corresponding document

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